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(54) Title: MONOCLONAL ANTIBODIES AND THEIR USE

(57) Abstract

Monoclonal antibodies to the genus *Bacillus*, the labelled antibodies, compositions and kits containing them, and their use in diagnosis of antigen and treatment.

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MONOCLONAL ANTIBODIES AND THEIR USE

BACKGROUND OF THE INVENTION

Of current interest in the fields of analysis and diagnosis is the use of monoclonal antibodies to determine the presence of antigens or species in specimens such as urine, blood, water, milk, and the like.

More particularly, monoclonal antibodies specific for the antigens or species of Bacillus are desired which when used will rapidly diagnose the presence of such organisms in specimens.

Divisions have been made among the Bacillus species. Some of the representative members which have been characterized as causing diseases in certain circumstances include Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Bacillus anthracis and Bacillus licheniformis.

Bacillus will be described with particular reference to Bacillus cereus. The Bacillus cereus spore is known to cause a special form of food poisoning associated with the ingestion of rice. This spore is resistant to heat and in routine cooking survives destruction. If rice is maintained in restaurants at a slightly warmer temperature to retain its fluffiness, the bacillus spore will germinate and produce a toxin which produces severe gastrointestinal

upset, nausea, vomiting, and diarrhea. This toxin may occur some hours after ingestion of the toxin. Bacillus cereus is known to produce four toxins: cereolysin, diarrheagenic, emetic, and enterotoxins. Bacillus subtilis causes the subtilysin toxin, and Bacillus anthracis produces an anthracis toxin.

Present detection of toxin contamination in food utilizes rather cumbersome methodology. Thus, the use of rapid diagnostic methods in the food technology and the public health industry would greatly expand with simpler and faster tests. The ability of monoclonal antibodies specifically to bind to antigens of Bacillus or Bacillus toxin can provide many opportunities for diagnosis and treatment. Such specificity is a most important requirement for proper and accurate analysis and/or diagnosis, particularly in diagnosing the presence of diseases which require prompt treatment.

A wide variety of isotopic and nonisotopic immunoassays have been utilized in conjunction with monoclonal antibodies to test for the presence of an antigenic substance. At the present time, agglutination, immuno-fluorescent, chemiluminescent or fluorescent immunoassay, immuno-

electron microscopy, radiometric assay systems, radio immunoassays, and enzyme-linked immunoassays are the most common techniques used with the monoclonal antibodies. Other techniques include bioluminescent, fluorescence polarization, and photon-counting immunoassays.

When utilizing the enzyme-linked immunoassay procedure (EIA), it is necessary to bind, or conjugate, the monoclonal antibody with an enzyme capable of functioning in such assay; such as alkaline phosphatase.

The enzyme-linked monoclonal antibody can then be used in the known enzyme-linked immunosorbent assay procedure to determine the presence of an antigenic substance.

After the specific antigen is identified, the serotype of the infecting organism can be determined, and appropriate treatment can then be initiated to rapidly and efficiently eliminate the disease.

The production of monoclonal antibodies is now a well-known procedure first described by Kohler and Milstein (Eur. J. Immunol. 6, 292 (1975)). While the general technique of preparing hybridomas and the resultant monoclonal antibodies is understood, it has been found

that preparing a specific monoclonal antibody to a specific antigen is difficult, mainly due to the degree of specificity and variations required in producing a particular hybridoma.

SUMMARY OF THE INVENTION

The present invention provides novel monoclonal antibodies for use in accurately and rapidly diagnosing samples for the presence of Bacillus antigens and/or organisms.

Briefly stated, the present invention comprises monoclonal antibodies specific for an antigen or species of Bacillus; in particular, the antigens or species of Bacillus cereus, and the antigens or species of Bacillus megaterium, Bacillus subtilis, Bacillus anthracis, the antigen or antigens for the toxins of Bacillus cereus, B. megaterium, B. subtilis, and B. anthracis, as well as a monoclonal antibody broadly cross-reactive with an antigen for each species of the genus Bacillus.

The invention also comprises labeled monoclonal antibodies for use in diagnosing the presence of the Bacillus antigens, each comprising a monoclonal antibody against one of the above-mentioned antigens to Bacillus or to a particular species or toxins thereof and

linked thereto an appropriate label. The label can be chosen from the group consisting of a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle, or any other label.

The invention further comprises the process for diagnosing the presence of Bacillus antigens, organisms, or toxins in a specimen comprising contacting said specimen with the labeled monoclonal antibody in an appropriate immunoassay procedure.

Additionally, the invention is also directed to a therapeutic composition comprising a monoclonal antibody for an antigen or toxin of Bacillus and a carrier or diluent, as well as kits containing at least one labeled monoclonal antibody to an antigen or toxin of Bacillus.

DETAILED DESCRIPTION

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against the particular Bacillus antigen, with an appropriate myeloma cell line, preferably NS0 (uncloned), P3NS1-Ag4/1, or Sp2/0 Ag14. The resultant product is then cultured in a standard HAT (hypoxanthine,

aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is first immunized by injection of the particular Bacillus antigen chosen generally for a period of approximately eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the appropriate Bacillus antigen, and then killed so that the immunized spleen may be removed. The fusion can then be carried out utilizing immunized spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the particular Bacillus antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine

their specificity for the particular Bacillus antigen. The monoclonal antibody selected, which is specific for the particular Bacillus antigen, species, or toxin, is then bound to an appropriate label.

Amounts of antibody sufficient for labeling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labeled with a multitude of different labels, such as enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labeled monoclonal antibody. Some of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, or urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labeled monoclonal antibody is

formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (EIA). EIA is a solid phase assay system which is similar in design to the radiometric assay, but which utilizes an enzyme in place of a radioactive isotope as the immunoglobulin marker.

Fluorescent-immunoassay is based on the labeling of antigen or antibody with fluorescent probes. A nonlabeled antigen and a specific antibody are combined with identical fluorescently labeled antigen. Both labeled and unlabeled antigen compete for antibody binding sites. The amount of labeled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of nonlabeled antigen. Examples of this particular type of fluorescent-immunoassay would include heterogenous systems such as Enzyme-Linked Fluorescent Immunoassay, or homogeneous systems such as the Substrate

Labeled Fluorescent Immunoassay. The most suitable fluorescent probe, and the one most widely used is fluorescein. While fluorescein can be subject to considerable interference from scattering, sensitivity can be increased by the use of a fluorometer optimized for the probe utilized in the particular assay and in which the effect of scattering can be minimized.

In fluorescence polarization, a labeled sample is excited with polarized light and the degree of polarization of the emitted light is measured. As the antigen binds to the antibody its rotation slows down and the degree of polarization increases. Fluorescence polarization is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per liter range and upper nanomole per liter range with respect to antigens in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically

excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. The best known chemiluminescent substance is luminol.

A further aspect of the present invention is a therapeutic composition comprising one or more of the monoclonal antibodies to the particular Bacillus antigen, species, or toxin, as well as a pharmacologically acceptable carrier or diluent. Such compositions can be used to treat humans and/or animals afflicted with some form of Bacillus infections and they are used in amounts effective to cure; an amount which will vary widely dependent upon the individual being treated and the severity of the infection.

One or more of the monoclonal antibodies can be assembled into a diagnostic kit for use in diagnosing for the presence of antigens, toxins, or species of Bacillus in various specimens. For example, a rapid diagnostic method requiring limited technical skill could be

utilized in the detection of Bacillus cereus toxin in public health analysis of restaurant practices, food handling in the food technology industry, and as an emergency diagnostic in the emergency care of patients with acute gastrointestinal symptoms. It is also possible to use the broadly cross-reactive monoclonal antibody which can identify the genus Bacillus alone or as part of a kit containing antibodies that can identify other bacterial genera or species of Bacillus and/or other toxins.

In the past there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. A rapid and precise kit could replace or augment existing tests and permit early direct therapy using precise antibiotics. Avoiding multiple antibiotics or more expensive or hazardous antibiotics would represent substantial patient and hospital savings. Additionally, a kit can be used on an out-patient basis. At present the lack of a rapid test giving "same day" answers may delay the initiation of treatment until the patient

has developed more severe symptoms or may require the initiation of more costly therapy in a sick patient. A test that would return results within an hour or two would be a substantial convenience to patients.

In addition to being sold individually, the kit could be included as a component in a comprehensive line of compatible immunoassay reagents sold to reference laboratories to detect the species and serotypes of Bacillus.

One preferred embodiment of the present invention is a diagnostic kit comprising at least one labeled monoclonal antibody against a particular Bacillus antigen, toxin, or species, as well as any appropriate stains, counterstains, or reagents. Further embodiments include kits containing at least one control sample of a Bacillus antigen and/or a cross-reactive labeled monoclonal antibody which would detect the presence of any of the Bacillus organisms or toxins in a particular sample. Specific antigens to be detected in this kit include the antigens of Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Bacillus anthracis, as well as the antigen or antigens for the toxins of Bacillus cereus, B. megaterium, B. subtilis, and B.

anthracis.

Monoclonal diagnostics which detect the presence of Bacillus antigens can also be used in periodic testing of water sources, food supplies and food processing operations. Thus, while the present invention describes the use of the labelled monoclonal antibodies to determine the presence of a standard antigen, the invention can have many applications in diagnosing the presence of antigens by determining whether specimens such as urine, blood, stool, water and milk contain the particular Bacillus antigen. More particularly, the invention could be utilised as a public health and safety diagnostic aid, whereby specimens such as water or food could be tested for possible contamination.

The invention will be further illustrated in connection with the following Examples which are set forth for the purposes of illustration only and not by way of limitation.

In the Examples:

API = Analytical Profile Index (ref. Ayerst Labs)

DMEM = Dulbecco's Modified Eagles Medium

FCS = Foetal Calf Serum

PBS = phosphate-buffered saline

% T refers to vaccine concentration measured in a 1 cm light path

Monoclonal antibodies of the present invention are prepared generally according to the method of Koehler and Milstein, Eur. J. Immunol. 6, (1975) 292.

EXAMPLE 1

A. Antigen Preparation

Antigen (Bacillus cereus) is obtained (samples are available from the National Collection of Type Cultures) and tested by standard biochemical methods of microbial identification to confirm its identity (using API profiles). The antigen is removed from the lyophile,

grown on blood agar, and tested by API to confirm its identity and purity. It is then transferred for growth into DMEM. After incubation, the cells are held at 100°C for 1 hour, harvested by centrifugation, and washed three times in saline. They are then resuspended in 1% formol saline.

B. Animal Immunisation

Balb/c mice are injected with the prepared antigen. They are given intraperitoneal and/or intravenous injections (0.05 ml 80% T vaccine) of vaccine prepared as above. The mice are bled approximately six days after the last injection and the serum tested for antibodies by assay. A conventional assay used for this serum titer testing is the enzyme-linked immunosorbent assay system. When the mice show antibody production after this regimen, generally a positive titer of at least 10,000, a mouse is selected as a fusion donor and given a booster injection (0.02 ml 80% T vaccine) intravenously, three days prior to splenectomy.

C. Cell Fusion

Spleen cells from the immune mice are harvested three days after boosting, by conventional techniques. First, the donor mouse selected is killed and surface-sterilised by immersion in 70% ethyl alcohol. The spleen is then removed and immersed in approximately 2.5 ml DMEM to which has been added 3% FCS. The spleen is then gently homogenised in a LUX homogenising tube until all cells have been released from the membrane, and the cells are washed in 5 ml 3% FCS-DMEM. The cellular debris is then allowed to settle and the spleen cell suspension placed in a 10 ml centrifuge tube. The debris is then rewashed in 5 ml 3% FCS-DMEM. 50 ml suspension are then made in 3% FCS-DMEM.

The myeloma cell line used is NSO (uncloned), obtained from the MRC Laboratory of Molecular Biology in

Cambridge, England. The myeloma cells are in the log growth phase, and rapidly dividing. Each cell line is washed using, as tissue culture medium, DMEM containing 3% FCS.

The spleen cells are then spun down at the same time that a relevant volume of myeloma cells are spun down (room temperature for 7 minutes at 600 g), and each resultant pellet is then separately resuspended in 10 ml 3% FCS-DMEM. In order to count the myeloma cells, 0.1 ml of the suspension is diluted to 1 ml and a haemacytometer with phase microscope is used. In order to count the spleen cells, 0.1 ml of the suspension is diluted to 1 ml with Methyl Violet-citric acid solution, and a haemacytometer and light microscope are used to count the stained nuclei of the cells.

Spleen cells are then mixed with myeloma cells, the mixture washed in serum-free DMEM high in glucose, and centrifuged, and all the liquid removed. The resultant cell pellet is placed in a 37°C water-bath. 1 ml of a 50 w/v solution of polyethylene glycol 1500 (PEG) in saline Hepes, pH approximately 7.5, is added, and the mixture gently stirred for approximately 1.5 minutes. 10 ml serum-free tissue culture medium DMEM are then slowly added, followed by up to 50 ml of such culture medium, centrifugation and removal of all the supernatant, and resuspension of the cell pellet in 10 ml of DMEM containing 18% by weight FCS.

10 µl of the mixture are placed in each of 672 wells of standard multiwell tissue culture plates. Each well contains 1.0 ml of the standard HAT medium (hypoxanthine, aminopterin and thymidine) and a feeder layer of Balb/c macrophages at a concentration of 5×10^4 macrophages/well.

The wells are kept undisturbed, and cultured at 37°C in 9% CO_2 air at approximately 100% humidity. The wells

are analysed for growth, utilising the conventional inverted microscope procedure, after about 5 to 10 days. In those wells in which growth is present in the inhibiting HAT medium, screening tests for the specific monoclonal antibody are made utilising the conventional enzyme immunoassay screening method described below. Somewhere around 10 days to 14 days after fusion, sufficient antibody against the antigen may develop in at least one well.

D. Cloning

From those wells which yielded antibody against the antigen, cells are removed and cloned using the standard agar or dilution method.

The clones may be assayed by the enzyme immunoassay method to determine antibody production.

E. Monoclonal Selection

The monoclonal antibodies from the clones are screened by the standard techniques for binding to the antigen, prepared as in the immunisation, and for specificity in a test battery of the class bearing different antigens. Specifically, a grid of microtiter plates containing a representative selective of organisms is prepared, boiled, and utilised as a template to define the specificity of the parent group. The EIA immunoassay noted above may be used.

F. Antibody Production and Purification (2 alternatives

(1) Six Balb/c mice are primed with pristane and injected intraperitoneally with 10^7 cells of the monoclonal antibody specific against the antigen. The ascites fluid is harvested after the mice have reached the proper stage; the mice are swollen with fluid but still alive.

The cells are then centrifuged at 1200 g for approximately 10 minutes, the cells discarded, and the antibody-rich ascites fluid collected. The fluid is

titrated, as noted above, to establish presence and level of antibody, and purified.

Purification is accomplished using the protein A - Sepharose method. More particularly, about 10 ml of the ascites fluid are filtered through glass wool and centrifuged at 30,000 g for 10 minutes. The ascites is then diluted with twice its own volume of cold phosphate buffer (0.1 M sodium phosphate, pH 8.2). The diluted ascites is loaded on to a 2 ml column of protein A - Sepharose which has previously been equilibrated with phosphate buffer. The column is washed with 40 ml phosphate buffer, and the monoclonal antibody is eluted with citrate buffer (0.1 M sodium citrate, pH 3.5) into sufficient 1M tris buffer, pH 9.0, to raise the pH immediately to about 7.5. The eluate is dialysed in 2 x 1000 ml PBS at +4°C.

(2) Cells of the monoclonal antibody-producing line specific to Bacillus cereus are grown in batch tissue culture. DMEM, to which has been added 10% FCS, is used to support growth in mid-log phase, to 1 litre volume. The culture is allowed to overgrow, to allow maximum antibody production. The culture is then centrifuged at 1200 g for approximately 10 minutes. The cell/cell debris is discarded and the antibody-rich supernatant collected.

The fluid may then be titrated, as noted above, to establish presence and level of antibody, and purified by a combination of batch ion-exchange chromatography, ammonium sulphate precipitation and column ion-exchange (a possible alternative would be protein A - Sepharose) chromatography.

More particularly, to one litre of culture supernatant is added one litre of 0.05M sodium acetate buffer, pH 4.5, and 40 ml of SP-Sephadex, previously equilibrated in 0.1M sodium acetate buffer, pH 5.0. The

suspension is stirred at +4°C for one hour. The SP-Sephadex is allowed to settle and the supernatant is decanted. The SP-Sephadex is packed in a column, washed with 60 ml of 0.1M acetate buffer, pH 5.0, and eluted with 60 ml of the same buffer plus 1M sodium chloride. The eluate is stirred at +4°C, and an equal volume of saturated ammonium sulphate added slowly. The suspension is stirred for a further 30 minutes. The precipitate is then harvested by centrifugation at 10,000 g for 10 minutes. The precipitate is dissolved in a minimum volume of either cold phosphate/EDTA buffer (20mM sodium phosphate, 10mM EDTA, pH 7.5, + 0.02% sodium azide) for DEAE-cellulose chromatography, or phosphate buffer (0.1M sodium phosphate, pH 8.2 + 0.02% sodium azide) for protein A-Sepharose chromatography. The dissolved precipitate is dialysed versus 2 x 1000 ml of the dissolution buffer at +4°C, and the appropriate chromatography step carried out as previously described.

G. Enzyme-Monoclonal Linkage

The monoclonal antibody specific against the antigen, prepared as above, is linked to an enzyme, viz. highly-purified alkaline phosphatase. The one-step glutaraldehyde method or benzoquinone conjugation is used.

In the one-step glutaraldehyde method, 3 mg monoclonal antibody (in about 1 ml of solution) are dialysed with 10 mg alkaline phosphatase (Sigma Type VII-T) against 2 x 1000 ml of PBS, pH 7.4, at +4°C. After dialysis, the volume is made up to 2.5 ml with PBS, and 25 µl of a 20% glutaraldehyde in PBS solution are added. The conjugation mixture is left at room temperature for 1.5 hours. After this time, glutaraldehyde is removed by gel filtration on a Pharmacia PH-10 (Sephadex G-25 M) column, previously equilibrated in PBS. The conjugate is eluted with 3.5 ml

PBS and then dialysed against 2 x 2000 ml of TRIS buffer (50 mM TRIS, 1 mM magnesium chloride, pH 8.0, plus 0.02% sodium azide) at +4°C. To the dialysed conjugate is added 1/10th its own volume of 10% BSA in TRIS buffer. The conjugate is then sterile-filtered through a 0.22 µm membrane filter into a sterile amber vial and stored at +4°C.

EXAMPLE 2

The procedure of Example 1 may be followed to produce a monoclonal antibody against Bacillus cererus diarrheal toxin. The antigen is prepared by brain-heart infusion (1% glucose), and the supernatant filtered.

EXAMPLE 3

The general procedure of Example 1 may be followed to produce a monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Bacillus.

Tests using the present invention are superior to existing tests, based on the following advantages: (i) greater accuracy; (ii) same day results, within an hour or two; (iii) reduction in amount of skilled labour required to administer laboratory procedures, resulting in reduced labour costs; (iv) reduction in laboratory time and space used in connection with tests, resulting in reduced overhead expenses; and (v) improved therapy based upon early, precise diagnosis.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody specific for an antigen or species of Bacillus.

2. The antibody of Claim 1 specific to the antigen or antigens of Bacillus cereus.

3. The antibody of Claim 1 specific to the antigen or antigens of Bacillus cereus toxin.

4. The antibody of Claim 1 specific to the antigen or antigens of the Bacillus cereus toxin cereolysin.

5. The antibody of Claim 1 specific to the antigen or antigens of the Bacillus cereus diarrheagenic toxin.

6. The antibody of Claim 1 specific to the antigen or antigens of the Bacillus cereus emetic toxin.

7. The antibody of Claim 1 specific to the antigen or antigens of the Bacillus cereus enterotoxins.

8. The antibody of Claim 1 specific to the antigen or antigens of Bacillus subtilis.

9. The antibody of Claim 1 specific to the antigen or antigens of Bacillus subtilis toxins.

10. The antibody of Claim 1 specific to the antigen or antigens of the Bacillus subtilis subtilysin toxin.

11. The antibody of Claim 1 specific to the antigen or antigens of Bacillus megaterium.

12. The antibody of Claim 1 specific to the antigen or antigens of Bacillus anthracis.

13. The antibody of Claim 1 specific to the antigen or antigens of Bacillus anthracis toxin.

14. A monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Bacillus.

15. A labeled monoclonal antibody consisting

essentially of a monoclonal antibody of Claims 1-14 and an appropriate label.

16. The labeled monoclonal antibody of Claim 15, wherein said label is a member of the group selected from a radioactive isotope, enzyme, fluorescent compound, bioluminescent compound, chemiluminescent compound, or ferromagnetic atom, or particle.

17. The labeled monoclonal antibody of Claim 16, wherein said label is an enzyme capable of conjugating with a monoclonal antibody and of being used in an enzyme-linked immunoassay procedure.

18. The labeled monoclonal antibody of Claim 17, wherein said enzyme is alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

19. The labeled monoclonal antibody of Claim 16, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent immunoassay, or

fluorescence polarization immunoassay, photon counting immunoassay, or the like procedure.

20. The labeled monoclonal antibody of Claim 19, wherein said fluorescent compound or probe is fluorescein.

21. The labeled monoclonal antibody of Claim 16, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

22. The labeled monoclonal antibody of Claim 21, wherein such chemiluminescent compound is luminol or a luminol derivative.

23. The labeled monoclonal antibody of Claim 16, wherein said label is a bioluminescent compound capable of being used in an appropriate bioluminescent immunoassay.

24. The labeled monoclonal antibody of Claim 23, wherein such bioluminescent compound is luciferase or a luciferase derivative.

25. A process for diagnosing for the pre-

sence of an antigen of Bacillus in a specimen comprising contacting at least a portion of said specimen with a labeled monoclonal antibody of Claim 15 in an immunoassay procedure appropriate for said label.

26. The process of Claim 25, wherein the appropriately labeled immunoassay procedure is selected from immuno-fluorescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, enzyme-linked immunoassays, fluorescence polarization, photon-counting bioluminescent, or chemiluminescent immunoassay.

27. The process of Claim 26, wherein said label is an enzyme capable of being used in an enzyme-linked immunoassay procedure.

28. The process of Claim 27, wherein said enzyme is selected from alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

29. The process of Claim 26, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent

immunoassay, or fluorescence polarization immunoassay, or photon-counting immunoassay, or the like procedure.

30. The process of Claim 29, wherein said fluorescent compound or probe is fluorescein.

31. The process of Claim 26, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

32. The process of Claim 31, wherein said chemiluminescent compound is luminol or a luminol derivative.

33. The process of Claim 26, wherein said label is a bioluminescent compound capable of being used in a bioluminescent or enzyme-linked bioluminescent immunoassay.

34. The process of Claim 33, wherein said bioluminescent compound is luciferase or a luciferase derivative.

35. A therapeutic composition comprising

one or more of the monoclonal antibodies in Clims 1-14 and a pharmaceutically acceptable carrier or diluent.

36. A therapeutic composition comprising one or more of the labeled monoclonal antibodies in Claim 15 and a pharmaceutically acceptable carrier or diluent.

37. A method of treating Bacillus infections comprising administering an effective amount of a monoclonal antibody of Claims 1-14.

38. A kit for diagnosing for the presence of an antigen or species of Bacillus in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-14.

39. The kit of Claim 38, wherein said at least one antibody is labeled.

40. The kit of Claim 39, wherein said at least one monoclonal antibody is labeled with a fluorescent compound.

41. The kit as in Claim 39, wherein said

at least one monoclonal antibody is labeled with an enzyme.

42. The kit as in Claim 39, wherein said at least one monoclonal antibody is labeled with a member of the group consisting of a radioactive isotope, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle.

43. The kit of Claims 39, 40, 41, and 42 additionally containing at least one known Bacillus antigen as a control.

44. The kit of Claims 39, 40, 41, 42, and 43 containing each known antigen of Bacillus cereus, Bacillus megaterium, Bacillus subtilis, and Bacillus anthracis.

45. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of toxins of Bacillus cereus, Bacillus subtilis, Bacillus megaterium, and Bacillus anthracis.

46. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of Bacillus cereus.

47. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of Bacillus subtilis.

48. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of Bacillus megaterium.

49. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of Bacillus anthracis.

50. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of toxins of Bacillus cereus.

51. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of toxins of Bacillus subtilis.

52. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of toxins of Bacillus megaterium.

53. The kit of Claims 39, 40, 41, 42, and 43 containing the antigens of toxins of Bacillus anthracis.

54. A kit for diagnosing for the presence of an antigen or species of Bacillus in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-14 and a control.

55. The kit of Claim 54, wherein said at least one antigen is labeled and said control is at least one known antigen of Bacillus.

56. A kit for diagnosing for the presence of a Bacillus infection comprising at least one monoclonal antibody of Claims 1-14.

57. The kit of Claim 56, wherein said at least one monoclonal antibody is labeled.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00474

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 K 15/00; C 12 P 21/00; G 01 N 33/577; 33/569; A 61 K 39/40 // C 12 N 15/00 (C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System [8]	Classification Symbols
IPC[4]	C 12 P; G 01 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [9]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Biological Abstracts, vol. 80, no. 2, issued in 1985 (Philadelphia, PA, US) F. Shang et al.: "The monoclonal antibody against protective antigen from Bacillus anthracis: 1. Establishment of hybridoma cell lines.", see page 430, abstract no. 13029 & Acta Genet Sin 11(4): 265-269, 1984	1,12,13,38, 54-56
Y		15-34,39-53, 57
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X	Experientia, vol. 38, no. 9, issued September 1982 (Basel, CH) M. Huber-Lukac et al.: "Monoclonal antibodies against functionally distinct sites on the delta-endotoxin of Bacillus thuringiensis var. thuringiensis", see pages 1103-1105, especially page 1103, the summary	1,38,54-56
Y		15-34,39-53, 57
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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
5th February 1986	28 FEV. 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	EP, A, 0105804 (THE UNIVERSITY OF ROCHESTER) 18 April 1984 see claims 1-3,42,45,46,50; page 23, lines 10-27; page 24, table I; page 26, lines 10-27; page 32, line 17 - page 33, line 12	1,12-14,35, 36,38,54-56
Y		2-11,15-34, 39-53,57
	--	
Y	Chemical Abstracts, vol. 100, no. 15, issued 9 April 1984 (Columbus, Ohio, US) N.E. Thompson et al.: "Isolation and some properties of an enterotoxin	./.

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 37, because they relate to subject matter not required to be searched by this Authority, namely:

See Rule 39.1.iv. PCT

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	produced by Bacillus cereus", see page 169, abstract no. 116178b, & Infect. Immun. 1984, 43(3), 887-94 --	2-7,15-34,39-53,57
Y	Microbiology Abstracts, section B, vol. 6, no. 4, issued January 1971 (London, GB) A.W. Bernheimer et al.: "Nature and properties of a cytolytic agent produced by Bacillus subtilis", see page 16, abstract no. B2674; & J. Gen. Microbiol., 61, 361-369(1970) --	8-10,15-34,39-53,57
Y	Experientia, vol. 25, no. 3, issued 15 March 1969 (Basel, CH) A. Nacci et al.: "Antigenic components of germinated spores of Bacillus megaterium", see pages 302-303, especially page 303, right-hand column, lines 12-15 and 45-48 --	11,15-34,39-53,57
Y	US, A, 4461829 (A.C. GREENQUIST) 24 July 1984 see claims 1-3; column 2, lines 8-40; column 10, lines 17-26 and 51	15-34,39-53,57

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00474 (SA 10956)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 20/02/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0105804	18/04/84	None	
US-A- 4461829	24/07/84	CA-A- 1190461	16/07/85